

Applicants: Ilan Sela and Sylvia Zeitoune-Simovich
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In the claims:

Please replace the claims with the listing of claims below.

--1. (currently amended) An expression silencing system comprising:

- a) a first DNA construct comprising ~~a nucleotide sequence corresponding to~~ the T7 RNA polymerase gene (T7-pol) ~~or to a functional equivalent or fragment thereof which sequence carries and a~~ [[an]] NLS sequence, and further comprising at least one promoter and at least one terminator sequence operably linked to said T7-pol; and
- b) a second DNA construct comprising a T7 promoter sequence (pT7) ~~or a functional fragment thereof,~~ at least one targeting sequence downstream to said pT7 and at least one 3' non-translated terminator sequence operably linked to said targeting sequence;

which system is capable, upon introduction thereof into a cell, of rendering the expression at the RNA level of a target sequence in said cell, in a tissue or organ regenerated from said cell or in a progeny thereof, ~~substantially~~ silenced, by causing the ~~substantial disappearance of the RNA or RNA transcripts carrying said sequence or a functional part thereof of said target sequence.~~

--2. (currently amended) A protein expression silencing system comprising ~~a nucleotide sequence corresponding to~~ the T7 RNA polymerase gene (T7-pol) ~~or a functional equivalent or fragment thereof which~~

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~~sequence carries~~ and a ~~[[an]]~~ NLS sequence, which ~~construct further comprises~~ at least one promoter and at least one terminator sequence operably linked to said T7-pol, a T7 promoter (pT7) ~~or a functional equivalent or fragment thereof~~, at least one targeting sequence downstream to said ~~the~~ pT7, and at least one additional terminator sequence operably linked to said targeting sequence, said system being capable, upon introduction thereof into a cell, of rendering the expression at the RNA level of a target sequence in said cell, in a tissue or organ regenerated from said cell or in a progeny thereof, ~~substantially silenced~~, by causing the ~~substantial~~ disappearance of the RNA transcript carrying said target sequence.--

- 3. (currently amended) The expression silencing system as claimed in claim 1 or claim 2, wherein said cell, ~~in which the expression at the RNA level is substantially silenced~~ is ~~[[an]]~~ a eukaryotic cell or a prokaryotic cell selected from a plant cell, a mammalian cell, a bacterium, a yeast, their pathogens, or any suitable tissue culture cell.--
- 4. (original) The expression-silencing system as claimed in claim 1 or claim 2, wherein said regenerated organ is a flowering differentiated plant regenerated from said cell.--
- 5. (currently amended) The expression silencing system as claimed in claim 1 or claim 2, wherein said at least one targeting sequence is ~~substantially~~

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identical ~~or homologous~~ to at least part of said target sequence.--

--6. (currently amended) The expression silencing system as claimed in claim 5, wherein said target sequence ~~corresponds to~~ is:

- a) a gene encoding a protein or a peptide product, ~~the silencing of which is desired;~~
- b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence; or
- c) a nucleic acid sequence which ~~corresponds to~~ is (a) or (b) or ~~to a fragment thereof, within the scope of degeneracy of, the genetic code; or~~
- d) ~~a nucleic acid sequence which hybridizes with the sequence according to (a), to (b), or to (c) or with fragments thereof, which hybridization is carried out under conditions which allow such hybridization to occur.--~~

--7. (original) The expression silencing system as claimed in claim 6, wherein said gene encodes a plant protein or peptide product or a protein or peptide product of a plant pathogen.--

--8. (withdrawn) The expression silencing system as claimed in claim 7, wherein said protein or peptide product of a plant pathogen is plant virus, a bacterium or a fungus capable of infecting said plant.--

--9. (original) The expression silencing system as

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claimed in claim 7, wherein said gene encodes the GUS protein.--

- 10. (withdrawn) The expression silencing system as claimed in claim 6, wherein said gene encodes a human protein or peptide product or a protein or peptide product of a human pathogen.--
- 11. (original) The expression silencing system as claimed in claim 6, wherein said non-coding sequence is a regulatory element sequence which, under normal conditions, promotes the expression of a coding sequence.--
- 12. (withdrawn) The expression silencing system as claimed in claim 10, wherein said target sequence is the TMV non-coding sequence Ω .--
- 13. (currently amended) The expression silencing system as claimed in claim 5, ~~optionally~~ further comprising additional regulatory elements.--
- 14. (withdrawn) The expression silencing system as claimed in claim 5, wherein said NLS sequence is the SV-40 NLS sequence.--
- 15. (currently amended) The expression silencing system as claimed in claim 5, wherein said promoter sequence is ~~the plant~~ p35S promoter ~~p35S~~.--
- 16. (currently amended) The expression silencing system as claimed in claim 1 or claim 2, wherein said

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terminator is the NOS terminator or a ~~functional equivalent or a fragment thereof~~, the β -1,3-gluconase terminator, ~~or any other suitable terminator~~ capable of terminating the transcription of a nucleic acid sequence and of the addition of polyadenylated ribonucleotides to the 3' end of the primary transcript of said target sequence.--

--17. (currently amended) The expression silencing system as claimed in claim 1 or claim 2, wherein said pT7 ~~corresponds to~~ is the promoter region of the bacteriophage T7 ~~or functional analogues thereof~~, which promoter is capable of initiating transcription of said at least one targeting sequence downstream thereto.--

--18. (original) The expression silencing system as claimed in claim 1 or claim 2, comprising the T7 terminator and the NOS terminator operably linked to said targeting sequence.--

--19. (currently amended) The expression silencing system as claimed in claim 1, wherein said first and second DNA constructs are ~~substantially~~ as shown in Figures 1A and 1B, respectively.--

--20. (withdrawn) A process for the transformation of a plant with a gene-silencing system which process comprises:

a) transforming plant cells with:

i) a first DNA construct comprising a nucleotide sequence corresponding to the T7 RNA

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polymerase gene (T7-pol) or a functional equivalent or fragment thereof, at least one plant promoter and at least one plant terminator sequence operably linked to said T7-pol; and with

ii) a second DNA construct comprising a T7 promoter sequence, a targeting sequence downstream to said T7 promoter, and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence;

b) selecting the plant cells transformed with at least one DNA construct according (a) and regenerating said selected cells to provide a differentiated flowering plant.--

--21. (currently amended) A process for the transformation of a plant with a gene-silencing system, which process comprises:

a) transforming plant cells with a DNA construct comprising ~~a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries and~~ [[an]] a NLS sequence, ~~said sequence further comprising and~~ at least one plant promoter sequence and at least one plant terminator sequence operably linked to said polymerase gene, a T7 promoter sequence (pT7) ~~or a functional fragment thereof,~~ a targeting sequence downstream to said pT7, and

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at least one additional terminator sequence operably linked to said targeting sequence, which DNA construct is capable of silencing the expression of a target sequence in a plant transformed with said system or in progeny of said transformed plant; and

- b) selecting plant cells transformed with said DNA construct according to (a) and regenerating said selected cells to provide a differentiated flowering plant.--

--22. (currently amended) The process as claimed in claim 20 or claim 21, wherein said targeting sequence ~~substantially corresponds to~~ is identical said with said target sequence or to a fragment thereof.--

--23. (currently amended) The process as claimed in claim 22, wherein said target sequence ~~corresponds to~~ is:

- a) a gene encoding a protein or a peptide product
- b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence; or
- c) a nucleic acid sequence which ~~corresponds to~~ is (a) or (b) or a fragment thereof, ~~within the scope of degeneracy of the genetic code; or d)~~ a nucleic acid sequence which hybridizes with the sequence according to (a), to (b), or to (c) or with fragments thereof, which hybridization is carried out under conditions which allow such hybridization to occur.--

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--24. (currently amended) A method for producing a transgenic plant carrying a ~~substantially~~ silent target sequence, by hybridizing a plant carrying and expressing said target sequence with a transformed plant obtained by the process of claim 22.--

--25. (cancelled) --

--26. (currently amended) A transgenic plant or its progeny obtained by the method of claim 24 or claim 25, in which the expression of said target sequence is ~~substantially~~ suppressed.--

--27. (currently amended) A method of silencing the expression of a target sequence within the genome of a plant or within the genome of a plant infecting pathogen present in said cell plant, ~~prior to the following manipulation~~, which method comprises the steps of:

- a) providing a first plant capable of regenerating;
- b) hybridizing said first plant with a second plant double transformed with:
 - i) a first DNA construct comprising a ~~nucleotide sequence corresponding to~~ the T7 RNA polymerase gene (T7-pol) ~~or a functional equivalent or fragment thereof which sequence carries~~ and a [[an]] NLS sequence, said construct further comprising at least one plant promoter and at least one plant terminator sequence operably linked to said ~~sequence~~ T7-pol; and with

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- ii) a second DNA construct comprising a T7 promoter sequence (pT7), a targeting sequence downstream to said pT7 and a 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising additional regulatory elements operably linked to said targeting sequence; and
- c) selecting those plants obtained by the hybridization of step (b), in which the expression of said target sequence is substantially silenced.--

--28. (currently amended) A method of silencing the expression of a target sequence within the genome of a plant or within the genome of a plant infecting pathogen present in said ~~cell~~ plant prior to the following manipulation, which method comprises the steps of:

- a) providing a first plant comprising said target sequence, said plant being capable of regenerating;
- b) hybridizing said first plant with a second plant transformed with a DNA construct comprising ~~a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol), or a functional equivalent or fragment thereof which sequence carries an~~ a NLS sequence, said ~~construct further comprising~~ a plant promoter and a plant terminator sequence operably linked to said T7-pol, a T7 promoter (pT7) ~~or a functional fragment thereof,~~ a targeting

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sequence downstream to said pT7, and at least one additional promoter sequence operably linked to said targeting sequence; and

- c) selecting those plants obtained by the hybridization of step (b), in which the expression of said target sequence is ~~substantially~~ silenced.--

--29. (currently amended) A method as claimed in claim 27 or claim 28, wherein said targeting sequence ~~substantially corresponds to~~ is identical with said target sequence or ~~to~~ a fragment thereof.--

--30. (currently amended) The method as claimed in claim 28, wherein said target sequence ~~corresponds to~~ is:

a) a gene encoding a protein or a peptide product

b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence;

c) a nucleic acid sequence which ~~corresponds to~~ is (a) or (b) or a fragment thereof, ~~within the scope of degeneracy of the genetic code, or~~

d) ~~a nucleic acid sequence which hybridizes with the sequence according to (a), to (b), or to (c) or with fragments thereof, which hybridization is carried out under conditions which allow such hybridization to occur.--~~

--31. (withdrawn) A method of identifying a nucleic acid of interest within a plant's genome wherein said nucleic acid of interest encodes a pre-defined plant phenotype, which process comprises the steps of:

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- a) providing a first plant comprising within its genome said nucleic acid of interest;
- b) transforming said first plant with a second plant transformed with:
 - i) a first DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, said construct further comprising at least one plant promoter and at least one plant terminator sequence operably linked to said sequence;and with
 - ii) a second DNA construct comprising a T7 promoter sequence, a random nucleic acid sequence downstream to said T7 promoter, and a 3' non-translated terminator sequence operably linked to said random nucleic acid sequence, said construct optionally further comprising additional regulatory elements operably linked to said nucleic acid of interest, said transformation thus provides a population of transgenic plants;
- c) selecting from the population obtained in step (a) those transformed plants/plant cells in which the pre-defined phenotype is substantially silenced; and
- d) employing said random nucleic acid sequence within the genome of transformed plants selected in step (c) as a probe in screening genomic DNA and cDNA libraries of said first plant, thereby

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identifying the gene comprising said random nucleic acid sequence which gene is responsible for said pre-defined phenotype.--

- 32. (withdrawn) A method of identifying a nucleic acid of interest within a plant's genome wherein said nucleic acid of interest encodes a pre-defined plant phenotype, which process comprises the steps of:
- a) providing a first plant comprising within its genome said nucleic acid of interest;
 - b) transforming said first plant with a second plant transformed with a DNA construct comprising a nucleotide sequence corresponding to the T7 RNA polymerase gene (T7-pol) or a functional equivalent or fragment thereof which sequence carries an NLS sequence, said construct further comprising at least one plant promoter sequence and at least one plant terminator sequence operably linked to said T7-pol, said DNA construct further comprising a T7 promoter sequence or a functional fragment thereof, a random nucleic acid sequence downstream to said T7 promoter, and a 3' non-translated terminator sequence operably linked to said random nucleic acid sequence;
 - c) selecting from the plants obtained in step (b) transformed plants in which the pre-defined phenotype is substantially silenced; and
 - d) employing said random nucleic acid sequence within the genome of the transformed plants selected in step (c) as a probe in screening genomic DNA or cDNA libraries of said first

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plant, thereby identifying the gene comprising said random nucleic acid sequence, which gene is responsible for said pre-defined phenotype.--

--33. (new) A method for silencing the expression of a target gene within a plant cell comprising the steps of:

- a) transforming a plant cell with a first construct comprising the T7 RNA polymerase gene (T7-pol), a NLS sequence, and at least one promoter and at least one terminator sequence operably linked to said T7-pol;
- b) selecting plant cells transformed with said first DNA construct according to step (a);
- c) transforming the selected plant cells obtained in step (b) with a second DNA construct comprising a T7 promoter sequence, a targeting sequence downstream to said T7 promoter, and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence;
- d) selecting from the plant cells obtained in step (c), cells transformed with said second DNA construct;

whereby transformation of said plant cell with said first and second DNA constructs renders the expression of said target sequence silenced.--

--34. (new) The method as claimed in claim 33, wherein said target sequence is:

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- a) a gene encoding a protein or a peptide product
- b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence; or
- c) a nucleic acid sequence which is a fragment of (a) or (b).--

--35. (new) The method as claimed in claim 33, wherein said plant cell is a pathogen-infected cell and said target sequence is a gene of said pathogen or a fragment thereof, whereby transformation of said pathogen-infected plant cell with said first and second DNA constructs renders the expression of said pathogen target sequence silenced.--

--36. (new) The method as claimed in claim 33, wherein said target sequence is a gene of a plant infecting pathogen or a fragment thereof, whereby transformation of said plant cell with said first and second DNA constructs renders the expression of said pathogen target sequence in a plant subsequently infected with said pathogen silenced.--

--37. (new) A method for silencing the expression of a target gene within a plant cell comprising the steps of:

- a) transforming said plant cell with a DNA construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, said construct further comprising at least one plant promoter sequence and at least one plant terminator sequence operably linked to said T7 polymerase gene, a T7 promoter sequence (pT7), a targeting

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sequence downstream to said pT7, and at least one additional terminator sequence operably linked to said targeting sequence, which DNA construct is capable, upon transformation thereof into a plant cell, of rendering the expression of a target sequence in said plant cell silenced; and

- b) selecting plant cells transformed with said DNA construct according to (a) and regenerating said selected cells to provide a differentiated flowering plant.--

--38. (new) The method as claimed in claim 37, wherein said target sequence is:

- a) a gene encoding a protein or a peptide product
- b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence; or
- c) a nucleic acid sequence which is a fragment of (a) or (b).--

--39. (new) The method as claimed in claim 37, wherein said plant cell is pathogen-infected and said target sequence is a gene of said pathogen or a fragment thereof, whereby transformation of said plant cell with said DNA construct renders the expression of said pathogen target sequence silenced.--

--40. (new) A method for silencing the expression of a target gene within a plant comprising the steps of:

- a) transforming a first population of plant cells with a first construct comprising the T7 RNA

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polymerase gene (T7-pol) and a NLS sequence, and further comprising at least one promoter and at least one terminator sequence operably linked to said T7-pol;

- b) selecting the cells obtained in step (a), cells transformed with said first DNA construct, and regenerating said selected cells to provide a differentiated flowering plant; or
- c) transforming a second population of plant cells with a second DNA construct comprising a T7 promoter sequence, a targeting sequence downstream to said T7 promoter, and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence;
- d) selecting from the plant cells obtained in step (c), cells transformed with said second DNA construct, and regenerating said selected cells to provide a differentiated flowering plant;
- e) hybridizing a first plant transformed with said first DNA construct as obtained in (b), with a second plant transformed with said second DNA construct as obtained in (d), thereby providing a double-transformed plant in which the expression of said target gene is silenced.--

--41. (new) The method as claimed in claim 40, wherein said target sequence is:

- a) a gene encoding a protein or a peptide product

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- b) a non-coding nucleic acid sequence, which, under normal conditions, promotes the expression of an essential coding sequence;
- c) a nucleic acid sequence which is a fragment of (a) or (b).--

--42. (new) A process for the transformation of a plant with a gene-silencing system which process, comprises:

- a) transforming plant cells with:
 - i) first construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, and further comprising at least one promoter and at least one terminator sequence operably linked to said T7-pol;
 - and
 - ii) a second DNA construct comprising a T7 promoter sequence, a targeting sequence downstream to said T7 promoter, and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence;
- b) selecting from the cells obtained in (a), cells transformed with at least one of said DNA constructs (i) and (ii) and regenerating said selected cells to provide a differentiated flowering plant; and
- c) hybridizing a plant transformed with said DNA

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construct (i) with a plant transformed with said DNA construct (ii), thereby providing a double-transformed plant in which the expression of a target sequence is suppressed.-

--43. (new) The expression silencing system as claimed in claim 5, wherein said promoter sequence is a promoter functional in plants.--